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# ERUPTION OF PERMANENT DENTITION IN RHESUS MONKEYS EXPOSED TO ELF FIELDS

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April 1983

NAVAL AEROSPACE MEDICAL RESEARCH LABORATORY
PENSACOLA FLORIDA





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# ERUPTION OF PERMANENT DENTITION IN RHESUS MONKEYS EXPOSED TO ELF FIELDS

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Naval Medical Research and Development Command
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## SUMMARY PAGE

### THE PROBLEM

Before the construction of an extremely low frequency (ELF) submarine communications system it is essential to know the biological effects of the electric and magnetic fields of such a system. This study was designed to evaluate ELF field effects on the growth and development of the rhesus monkey starting at 30 days of age. A previous study at this laboratory initiated with young adult rhesus monkeys provided evidence that the ELF field-exposed males gained weight faster than control males during adolescence. The current study was initiated with very young animals to provide maximum opportunity to study growth relative to sex, endocrine function and metabolic effects. This report provides an analysis of dental maturation relative to ELF field exposure.

### FINDINGS

In comparing the eruption times of the first eight permanent teeth, no significant differences between study groups were evident. Teeth of the females consistently erupted at a slightly earlier age than the males. In comparing the exposed animals to the control animals the data did not provide evidence that exposure affected development of the permanent teeth.

### RECOMMENDATIONS

The intent of this interim report was to analyze available data for possible ELF exposure effects on the growth rate of the permanent teeth. The six-week examination intervals provided for the detection of obvious abnormalities. However, a study designed specifically to assess subtle alterations in tooth development and maturation would require more sophisticated methods of measurement and more frequent examinations.

### ACKNOWLEDGMENTS

The authors would like to acknowledge Dr. Gregory Lotz, Toby Griner, and Dr. Jay Pollack for their assistance with data analysis and preparation of this report.

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The animals used in this study were handled in accordance with the <u>Guide for the Care and Use of Laboratory Animals</u> prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animals Resources, National Research Council.

# INTRODUCTION

The first ELF exposure study conducted at this laboratory involved chronic exposure of rhesus monkeys to electric and magnetic fields over a three-year period. The study was initiated with young, feral male and female animals that had reached sexual maturity. ELF exposed males were found to gain weight at a significantly faster rate than control animals. Subsequently, a second study was initiated with 30 day old animals to further validate previous results and define the mechanism involved.

This interim report concerns the maturation of the permanent dentition in rhesus monkeys. Information dealing with tooth development is an adjunct to the primary emphasis of the study on endocrinology and somatic development. Partial data currently available provided eruption ages for the first four pairs of upper and lower permanent teeth. The purpose of this report is to note any difference in the eruption of permanent teeth between the group of animals exposed to ELF fields and the non-exposed group of animals.

For the purpose of analyzing the data the 60 animals were divided into four separate study groups. These groups were comprised of male and female, ELF exposed and unexposed animals. They are identified as (1) Control males, (2) Exposed males, (3) Control females, and (4) Exposed females. Two variables were considered in the analysis of the data; the sex of the animal and exposure to ELF.

Analysis for significant differences in the eruption ages of the eight permanent teeth (Shown in Tables 3-10) was accomplished by making the following comparisons between study groups:

Environmental (ELF) Influence

A. Control males vs. Exposed males

B. Control females vs. Exposed females

C. All control (male and female) vs. All exposed (male and female)

Sex Influence

D. Control males vs. Control females

E. Exposed males vs. Exposed females

F. All males (control and exposed) vs. all females (control and exposed).

Comparisons were analyzed statistically using Student's t-test. The experimental significance level was corrected for 48 multiple comparisons using the following formula (4):

$\alpha_E = 1-(1-\alpha_i)^k$ where:

$k$ = number of tests performed
$\alpha_i$ = .05 level of significance
$\alpha_E$ = corrected significance level

To achieve an overall significance level of .05, the calculated $\alpha_E$ is .001.

## PROCEDURE

Sixty rhesus monkeys (Macaca mulatta) were introduced into the ELF project at 30 days of age. To accomplish this, each newborn animal and the nursing mother were placed in the study when the infant reached one month of age. As in the first ELF study, the monkeys were housed in non-conducting Plexiglas(R) chambers (2). The animals were added to the study over a period of about 9 months. At weaning time nursing mothers were removed from the study and the young animals were housed in pairs at six months of age. The juvenile animals were separated into individual chambers as they reached 24 months of age. The sixty animals were evenly and randomly divided into control and ELF exposure groups. Each group of 30 animals consisted of 17 males and 13 females.

As part of the routine physical examination of the 60 monkeys used in the study, the eruption times of the teeth were charted at six week intervals. The earliest date that a tooth was first seen during a routine physical examination was indicated as its eruption time. This included the earliest time that any part of the tooth was seen cutting through the gingival surface. No distinction was made between eruption of the right or left tooth of a pair, consequently only one eruption time for each upper or lower pair of teeth was noted. The frequency of examination did not allow for accurate identification of the initial eruption of deciduous teeth. However, the six-week examinations did provide a reasonable evaluation for the eruption of the permanent dentition. Other authors have reported the eruption of permanent dentition in rhesus monkeys on the basis of one-tenth year increments (1,6). For the purpose of data analysis the animals in this study are represented in one-tenth year increments.

Because of the partial development of the permanent dentition at the time the data in this report were accumulated (1 September 1982), the eruption patterns of only four tooth types were considered; first and second molars, and first and second incisors. Each pair of teeth (left and right) are represented as follows:

Lower first molars . . . . . . LM1

Upper first molars . . . . . . UM1

Lower second molars . . . . . LM2

Upper second molars . . . . . UM2

Lower first incisors . . . . . LI1

Upper first incisors . . . . . UI1

Lower second incisors . . . . LI2

Upper second incisors . . . . UI2

All of the teeth being considered here were not yet present in some animals because of the eight month variation in age. This is reflected in the differences in sample size (n) represented in the data. The complete dentition of a mature male rhesus monkey is represented in Figure 1.

# RESULTS AND DISCUSSION

Evaluation of the eruption times for deciduous teeth was not attempted because of the examination interval. It has been suggested that weekly examinations are necessary to accurately chart the development of deciduous teeth (5). All 20 deciduous teeth (2 X $\frac{\text{iicmm}}{\text{iicmm}}$) emerge by 8.5 months of age. The sequence of emergence is as follows: all incisors, approximately 2.5 months; all canines and first molars, 4.5 months; and all second molars by 8.5 months. It is approximately a year after the emergence of all the deciduous teeth that the first permanent molars appear (3).

There is great variability in the eruption times of the permanent teeth between animals as reported in the literature (3). It may take twice as long for the permanent teeth to appear in some animals compared with other animals that have fast eruptive patterns. The first permanent molars appear at about 20 months of age and completion of the permanent dentition occurs with eruption of the third molars by about 8.5 years of age. The permanent dentition is comprised of 32 teeth ( 2 X $\frac{\text{IICPPMMM}}{\text{IICPPMMM}}$ ) (Figure 1). The approximate ages for the sequential eruption of the permanent teeth are as follows: first molars, 2 years of age; first incisors, 2.5 years of age; second incisors, 3 years of age; second molars, 3.5 years; canines and premolars, 4 years; and third molars by eight and one-half years (3). The third molars are reported to erupt later in females than in males. Tooth development and wear are of little value in assessing an animal's age beyond 7.5-8.5 years.

This report deals with animals of known age with a definite birth date. At the time the data in this report were compiled, the ages of the 60 animals ranged from 2.7 to 3.5 years with a mean age of 3.3 years and a standard deviation of $\pm$ .2 years.

Table 1 contains general statistical data used for comparison between study groups. These data are also represented as histograms in Figures 2-5 which show the frequency distribution of eruption times for teeth considered in this study. The frequency distribution for the mandibular teeth was superimposed over the maxillary histogram. This occurred because data first extracted from the records were on the mandibular teeth and they generally preceded the eruption of the maxillary teeth.

Table 2 provides a comparison of tooth eruption data from this study with tooth emergence data from Hartman and Straus (3). The mean emergence values taken from the literature were converted from months into tenths of a year analogous to the ELF data. The ELF project animals appear to have an earlier eruption age, particularly for the molars. The average difference in mean eruption ages for the two sets of data was about .35 years. This difference was probably due to reporting eruption age versus emergence age. The ELF eruption data represents first penetration of the tooth through the gingival surface, whereas emergence data represents complete exposure of the crown above the gingival surface. Data from the literature reported in Table 2 is very limited. However, other more comprehensive data (6) that were reported in graphic frequency distributions did not provide concise data values which could be used.

Tables 3-10 provide a comparison between study groups for the eruption ages of the first and second permanent incisors and the first and second permanent molars. Some of the specified teeth were not present in all animals due to the variation in age. Consequently, the degrees of freedom in each table do not always equate to a total of

60 animals. Two basic methods of comparison were used in each of the eight tables to isolate the possible effects of ELF exposure or sex. In each of the eight tables comparisons A, B, and C provide for the identification of possible environmental influence (ELF exposure vs. control) and comparisons D, E, and F provide for the identification of differences due to sex.

A consistent trend was noted in the mean eruption ages for each of the eight teeth between male and female animals (Tables 3-10). In each case the mean eruption ages were slightly less for the female groups compared to the males as has been noted in other macaques (7).

No significant differences were found between study groups at the .001 level which was the established criteria for the multiple t-tests. Therefore, it does not appear that an environmental effect due to ELF exposure influenced the eruption of the permanent teeth studied at this time. The age of eruption of these early permanent teeth in the ELF project animals is in general agreement with the age of emergence of the same teeth in other studies reported in the literature.

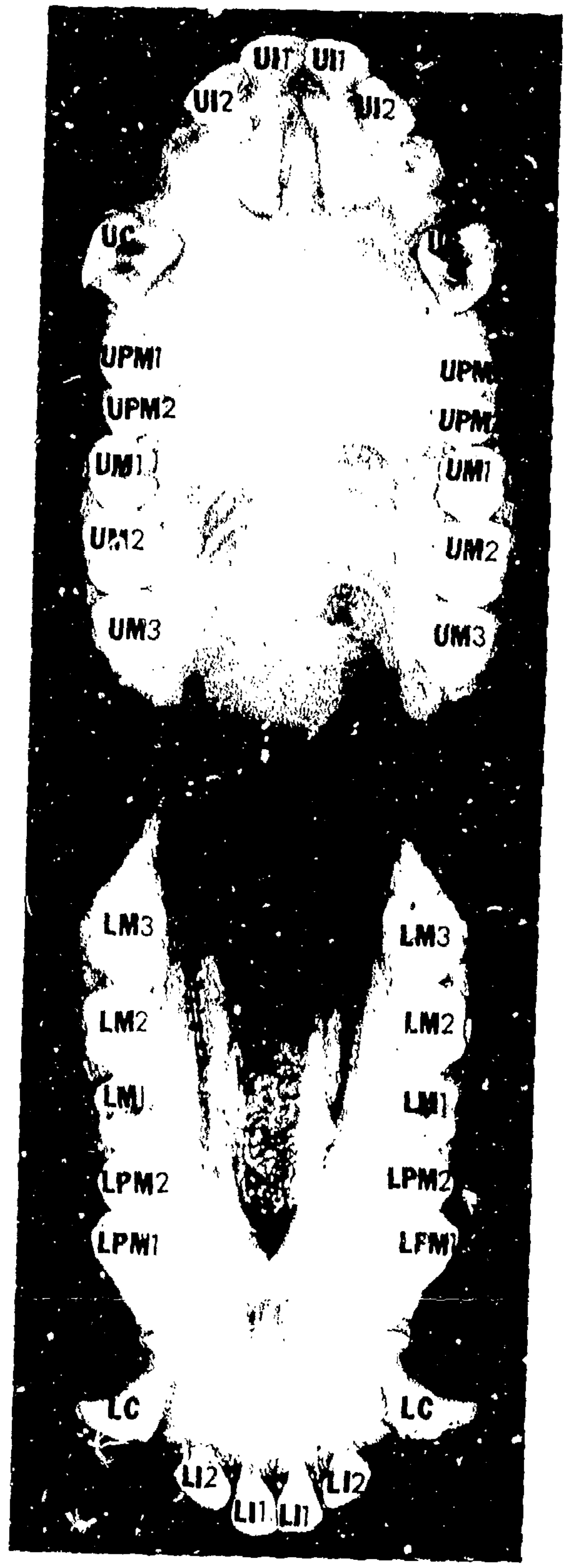


Figure 1. Permanent dentition of a mature male rhesus monkey.

FREQUENCY DISTRIBUTION OF ERUPTION TIMES FOR PERMANENT DENTITION
IN CONTROL MALES

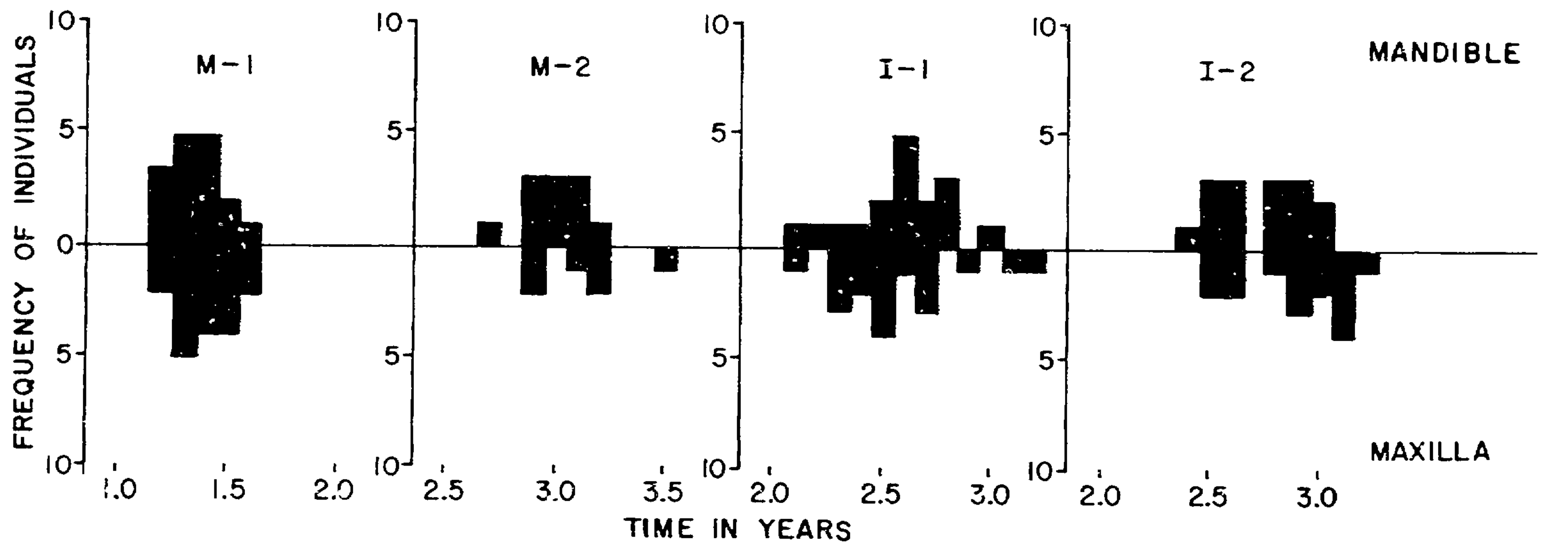


Figure 2

FREQUENCY DISTRIBUTION OF ERUPTION TIMES FOR PERMANENT DENTITION
IN EXPOSED MALES

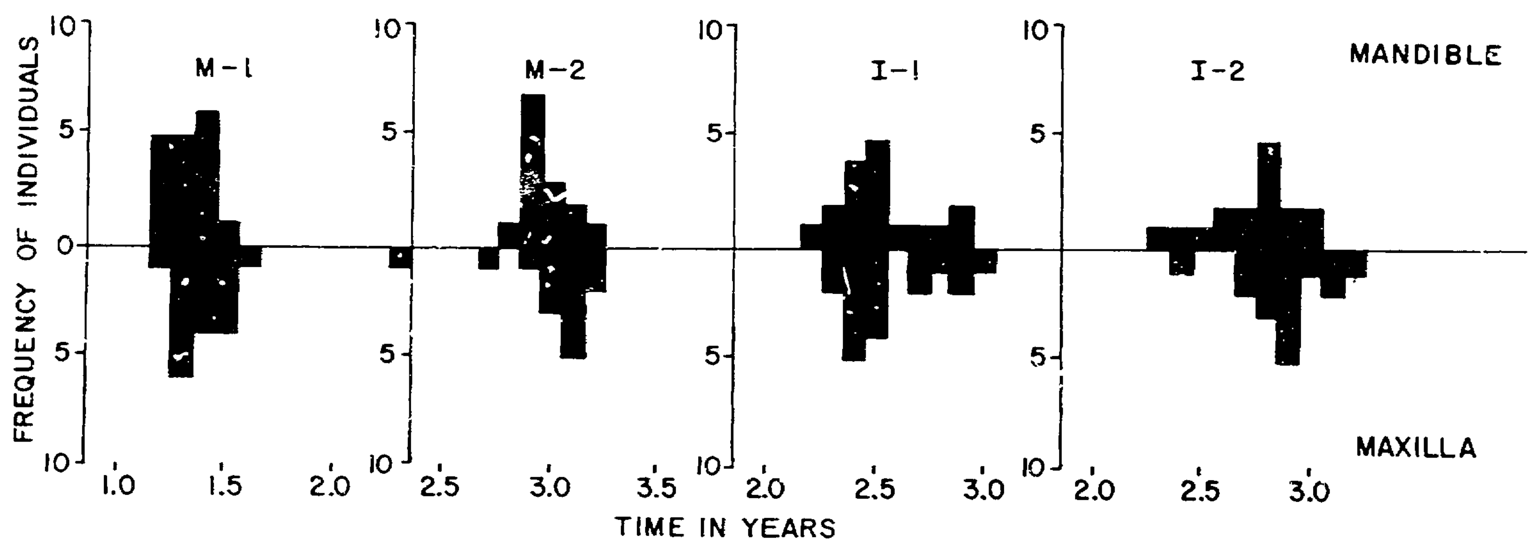


Figure 3

FREQUENCY DISTRIBUTION OF ERUPTION TIMES FOR PERMANENT DENTITION IN CONTROL FEMALES

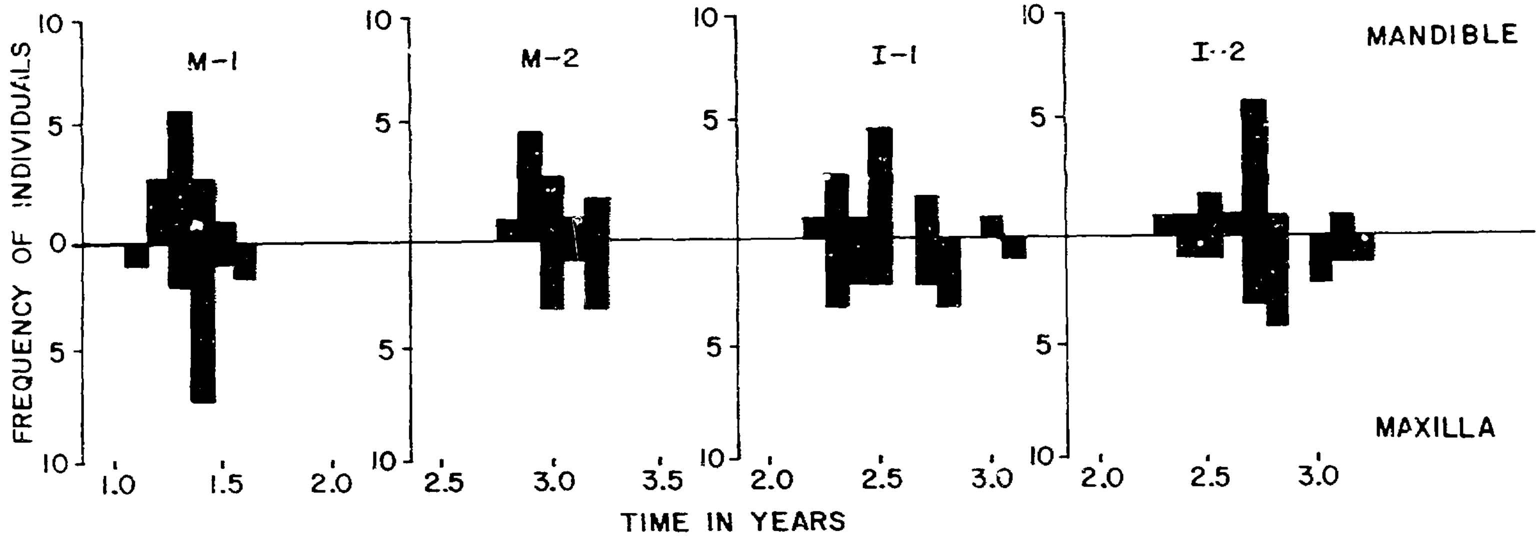


Figure 4

FREQUENCY DISTRIBUTION OF ERUPTION TIMES FOR PERMANENT DENTITION IN EXPOSED FEMALES

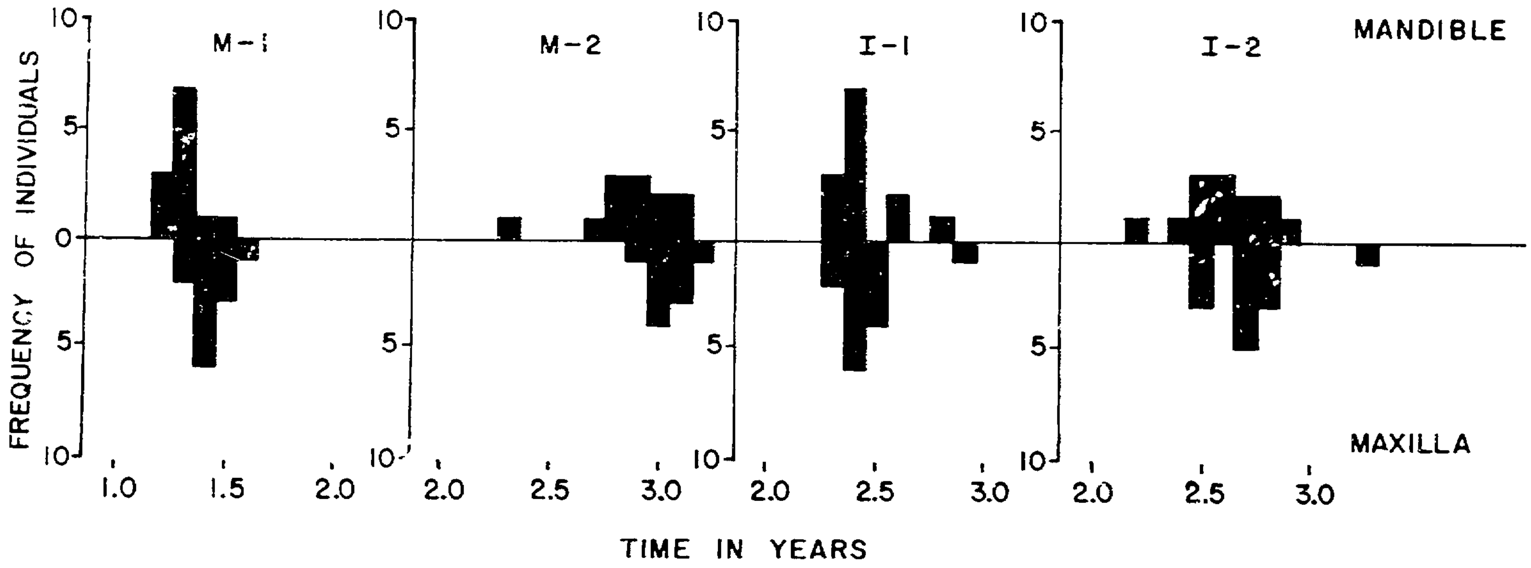


Figure 5

Table 1

Statistical Data on Control and Exposed ELF Monkey Tooth Eruption Times

| CONTROL MALES | N | MEAN* | RANGE | VARIANCE | STANDARD DEVIATION |
|---|---|---|---|---|---|
| LM1 | 16 | 1.36 | 1.2-1.6 | .01 | .12 |
| LM2 | 11 | 2.99 | 2.7-3.2 | .02 | .14 |
| LI1 | 17 | 2.58 | 2.1-3.0 | .05 | .23 |
| L12 | 15 | 2.72 | 2.4-3.0 | .04 | .20 |
| UM1 | 17 | 1.39 | 1.2-1.6 | .02 | .13 |
| UM2 | 6 | 3.13 | 2.9-3.5 | .05 | .23 |
| UI1 | 17 | 2.57 | 2.1-3.2 | .09 | .29 |
| UI2 | 15 | 2.89 | 2.5-3.2 | .06 | .24 |
| EXPOSED MALES | | | | | |
| LM1 | 17 | 1.32 | 1.2-1.5 | .01 | .10 |
| LM2 | 14 | 2.96 | 2.8-3.2 | .01 | .11 |
| LI1 | 17 | 2.52 | 2.2-2.9 | .04 | .20 |
| LI2 | 16 | 2.73 | 2.3-3.0 | .04 | .20 |
| UM1 | 17 | 1.44 | 1.2-2.3 | .06 | .25 |
| UM2 | 11 | 3.07 | 2.9-3.2 | .01 | .09 |
| UI1 | 17 | 2.57 | 2.3-3.0 | .05 | .22 |
| UI2 | 15 | 2.87 | 2.4-3.2 | .04 | .20 |
| CONTROL FEMALES | | | | | |
| LM1 | 13 | 1.32 | 1.2-1.5 | .01 | .09 |
| LM2 | 12 | 2.98 | 2.8-3.2 | .02 | .13 |
| LI1 | 13 | 2.49 | 2.2-3.0 | .05 | .21 |
| LI2 | 13 | 2.65 | 2.3-3.1 | .04 | .20 |
| UM1 | 13 | 1.40 | 1.1-1.6 | .02 | .13 |
| UM2 | 7 | 3.10 | 3.0-3.2 | .01 | .10 |
| UI1 | 13 | 2.59 | 2.3-3.1 | .06 | .25 |
| UI2 | 13 | 2.81 | 2.4-3.2 | .05 | .23 |
| EXPOSED FEMALES | | | | | |
| LM1 | 12 | 1.30 | 1.2-1.5 | .01 | .09 |
| LM2 | 12 | 2.86 | 2.3-3.1 | .05 | .22 |
| LI1 | 13 | 2.44 | 2.3-2.8 | .02 | .15 |
| LI2 | 13 | 2.60 | 2.2-2.9 | .04 | .19 |
| UM1 | 12 | 1.43 | 1.3-1.6 | .01 | .09 |
| UM2 | 9 | 3.04 | 2.9-3.2 | .01 | .09 |
| UI1 | 13 | 2.45 | 2.3-2.9 | .02 | .15 |
| UI2 | 13 | 2.71 | 2.5-3.2 | .03 | .19 |

*Tooth eruption times in years.

Table 2

Comparison of Tooth Eruption Times

| TOOTH | ELF PROJECT ANIMALS N | AGE OF ANIMAL IN YEARS* MEAN | MIN | MAX | DATA FROM HARTMAN & STRAUS (3) N | AGE OF ANIMAL IN YEARS** MEAN | MIN | MAX |
|---|---|---|---|---|---|---|---|---|
| LM1 | 58 | 1.3 | 1.2 | 1.6 | 9 | 1.7 | 1.5 | 2.0 |
| UM1 | 59 | 1.4 | 1.1 | 2.3 | 9 | 1.8 | 1.6 | 2.0 |
| LM2 | 49 | 2.9 | 2.3 | 3.2 | 5 | 3.5 | 3.2 | 4.3 |
| UM2 | 33 | 3.1 | 2.9 | 3.5 | 5 | 3.5 | 3.3 | 4.0 |
| LI1 | 60 | 2.5 | 2.1 | 3.0 | 8 | 2.8 | 2.3 | 3.0 |
| UI1 | 60 | 2.6 | 2.1 | 3.2 | 8 | 2.6 | 2.1 | 3.0 |
| LI2 | 57 | 2.7 | 2.2 | 3.1 | 7 | 3.0 | 2.8 | 3.4 |
| UI2 | 56 | 2.8 | 2.4 | 3.2 | 7 | 3.2 | 3.1 | 3.3 |

* Tooth eruption times noted when first signs of tooth breaking the gum were seen.

** Tooth eruption times noted when tooth had clearly emerged from the gum.

## Table 3

### Comparison of Mean Eruption Times for LM1

| | | $\mu$ | t | d.f. | prob.* |
|---|---|---|---|---|---|
| A | CONTROL MALES<br>EXPOSED MALES | 1.36<br>1.32 | 1.0520 | 31 | ns |
| B | CONTROL FEMALES<br>EXPOSED FEMALES | 1.32<br>1.30 | 0.4588 | 24 | ns |
| C | ALL CONTROL<br>ALL EXPOSED | 1.32<br>1.29 | 0.9801 | 58 | ns |
| D | CONTROL MALES<br>CONTROL FEMALES | 1.36<br>1.32 | 0.8050 | 28 | ns |
| E | EXPOSED MALES<br>EXPOSED FEMALES | 1.32<br>1.30 | 0.5131 | 27 | ns |
| F | ALL MALES<br>ALL FEMALES | 1.34<br>1.31 | 1.0949 | 56 | ns |

* $\alpha > .001$

Table 4

Comparison of Mean Eruption Times for UM1

| | | $\mu$ | t | d.f. | prob.* |
|---|---|---|---|---|---|
| A | CONTROL MALES<br>EXPOSED MALES | 1.39<br>1.44 | 0.7054 | 32 | ns |
| B | CONTROL FEMALES<br>EXPOSED FEMALES | 1.40<br>1.43 | 0.7165 | 24 | ns |
| C | ALL CONTROL<br>ALL EXPOSED | 1.40<br>1.41 | 0.2769 | 58 | ns |
| D | CONTROL MALES<br>CONTROL FEMALES | 1.39<br>1.40 | 0.1260 | 28 | ns |
| E | EXPOSED MALES<br>EXPOSED FEMALES | 1.44<br>1.43 | 0.3182 | 27 | ns |
| F | ALL MALES<br>ALL FEMALES | 1.42<br>1.41 | 0.1314 | 57 | ns |

* $\alpha$ · .001

Table 5

Comparison of Mean Eruption Times for LM2

| | | $\mu$ | t | d.f. | prob.* |
|---|---|---|---|---|---|
| A | CONTROL MALES<br>EXPOSED MALES | 2.99<br>2.96 | 0.5425 | 23 | ns |
| B | CONTROL FEMALES<br>EXPOSED FEMALES | 2.98<br>2.86 | 1.9875 | 23 | ns |
| C | ALL CONTROL<br>ALL EXPOSED | 2.99<br>2.92 | 1.6344 | 47 | ns |
| D | CONTROL MALES<br>CONTROL FEMALES | 2.99<br>2.98 | 0.1375 | 21 | ns |
| E | EXPOSED MALES<br>EXPOSED FEMALES | 2.96<br>2.86 | 1.8979 | 27 | ns |
| F | ALL MALES<br>ALL FEMALES | 2.98<br>2.92 | 1.2476 | 47 | ns |

* $\alpha$ = .001

Table 6

Comparison of Mean Eruption Times for UM2

| | | μ | t | d.f. | prob.* |
|---|---|---|---|---|---|
| A | CONTROL MALES | 3.13 | 0.7989 | 15 | ns |
| | EXPOSED MALES | 3.07 | | | |
| B | CONTROL FEMALES | 3.10 | 1.1798 | 14 | ns |
| | EXPOSED FEMALES | 3.04 | | | |
| C | ALL CONTROL | 3.12 | 1.2694 | 31 | ns |
| | ALL EXPOSED | 3.06 | | | |
| D | CONTROL MALES | 3.13 | 0.3550 | 11 | ns |
| | CONTROL FEMALES | 3.10 | | | |
| E | EXPOSED MALES | 3.07 | 0.7034 | 18 | ns |
| | EXPOSED FEMALES | 3.04 | | | |
| F | ALL MALES | 3.09 | 0.5830 | 31 | ns |
| | ALL FEMALES | 3.07 | | | |

* α < .001

Table 7

Comparison of Mean Eruption Times for LI1

| | | μ | t | d.f. | prob.* |
|---|---|---|---|---|---|
| A | CONTROL MALES | 2.58 | 0.7881 | 32 | ns |
| | EXPOSED MALES | 2.52 | | | |
| B | CONTROL FEMALES | 2.49 | 0.7519 | 24 | ns |
| | EXPOSED FEMALES | 2.44 | | | |
| C | ALL CONTROL | 2.54 | 1.0758 | 58 | ns |
| | ALL EXPOSED | 2.48 | | | |
| D | CONTROL MALES | 2.58 | 1.0216 | 32 | ns |
| | CONTROL FEMALES | 2.49 | | | |
| E | EXPOSED MALES | 2.52 | 1.1886 | 28 | ns |
| | EXPOSED FEMALES | 2.44 | | | |
| F | ALL MALES | 2.55 | 1.5530 | 58 | ns |
| | ALL FEMALES | 2.47 | | | |

* α ·.001

Table 8

Comparison of Mean Eruption Times for UI1

| | | μ | t | d.f. | prob.* |
|---|---|---|---|---|---|
| A | CONTROL MALES | 2.57 | 0.0661 | 32 | ns |
| | EXPOSED MALES | 2.57 | | | |
| B | CONTROL FEMALES | 2.59 | 1.6099 | 24 | ns |
| | EXPOSED FEMALES | 2.45 | | | |
| C | ALL CONTROL | 2.58 | 0.9779 | 58 | ns |
| | ALL EXPOSED | 2.52 | | | |
| D | CONTROL MALES | 2.57 | 0.1386 | 28 | ns |
| | CONTROL FEMALES | 2.59 | | | |
| E | EXPOSED MALES | 2.57 | 1.5384 | 28 | ns |
| | EXPOSED FEMALES | 2.45 | | | |
| F | ALL MALES | 2.57 | 0.7797 | 58 | ns |
| | ALL FEMALES | 2.52 | | | |

* α ·.001

Table 9

Comparison of Mean Eruption Times for LI2

| | | $\mu$ | t | d.f. | prob.* |
|---|---|---|---|---|---|
| A | CONTROL MALES<br>EXPOSED MALES | 2.72<br>2.73 | 0.0691 | 29 | ns |
| B | CONTROL FEMALES<br>EXPOSED FEMALES | 2.65<br>2.60 | 0.6103 | 24 | ns |
| C | ALL CONTROL<br>ALL EXPOSED | 2.69<br>2.67 | 0.3149 | 55 | ns |
| D | CONTROL MALES<br>CONTROL FEMALES | 2.72<br>2.65 | 0.9761 | 26 | ns |
| E | EXPOSED MALES<br>EXPOSED FEMALES | 2.73<br>2.60 | 1.7140 | 27 | ns |
| F | ALL MALES<br>ALL FEMALES | 2.72<br>2.62 | 1.9239 | 55 | ns |

* $\alpha$ .001

Table 10

Comparison of Mean Eruption Times for UI2

| | | μ | t | d.f. | prob.* |
|---|---|---|---|---|---|
| A | CONTROL MALES<br>EXPOSED MALES | 2.89<br>2.87 | 0.1690 | 28 | ns |
| B | CONTROL FEMALES<br>EXPOSED FEMALES | 2.81<br>2.71 | 1.2376 | 24 | ns |
| C | ALL CONTROL<br>ALL EXPOSED | 2.85<br>2.80 | 0.9207 | 54 | ns |
| D | CONTROL MALES<br>CONTROL FEMALES | 2.89<br>2.81 | 0.9025 | 26 | ns |
| E | EXPOSED MALES<br>EXPOSED FEMALES | 2.87<br>2.71 | 2.3007 | 26 | ns |
| F | ALL MALES<br>ALL FEMALES | 2.88<br>2.76 | 2.1691 | 54 | ns |

* $\alpha > .001$

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number):

In a study initiated to determine the biological effects of ELF electric and magnetic fields associated with a submarine communications system ELF-exposed male rhesus monkeys gained weight at a slightly faster rate that control males. In order to obtain sufficient data on the physiological effects of electromagnetic fields, a second ELF study was initiated. Whereas the first study was initiated with wild-caught young adult animals, the second study utilized colony-bred animals beginning at 30

(over)

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days of age. The emphasis of the second study was to substantiate previous findings and determine the underlying mechanisms involved. As in the first study, 30 primates (male and female) were exposed to the ELF electric and magnetic fields, and 30 control animals received the same care and treatment, but were not exposed. This report deals with the development of the permanent teeth relative to ELF exposure and sex. A consistent trend noted was that the teeth of female animals erupted at a slightly earlier age than males. However, no significant differences due to ELF exposure or sex were detected.

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